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13. ABSTRACT (Maximum 200 words)

The research program in our laboratory is directed at understanding the basic structure and function of the auditory system in fishes. The underwater environment is acoustically noisy and complex. Yet, fishes have evolved mechanisms to extract biologically relevant sounds from unimportant background stimuli. Experimental studies have demonstrated that extraction of signals from noise, discrimination between signals, and localizing the direction of sounds are very important to fishes. Significantly, fishes use an accelerometer-like ear and a relatively 'simple' central nervous system (CNS) to do this highly complex sound analysis. It is the objective of this study to understand how this accelerometer-like system is capable of complex signal analysis and processing. More specifically, we intend to determine auditory capabilities and mechanisms of fishes, the contributions of various parts of the auditory system (ear and peripheral structures) to signal analysis, and the neuroanatomy ('wiring') of the auditory portion of the CNS as it might contribute to signal analysis.

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ACOUSTIC TRANSDUCTION IN FISH
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RESEARCH GOALS

The research program in our laboratory is directed at understanding the basic structure and function of the auditory system in fishes. The underwater environment is acoustically noisy and complex. Yet, fishes have evolved mechanisms to extract biologically relevant sounds from unimportant background stimuli. Experimental studies have demonstrated that extraction of signals from noise, discrimination between signals, and localizing the direction of sounds are very important to fishes. Significantly, fishes use an accelerometer-like ear and a relatively 'simple' central nervous system (CNS) to do this highly complex sound analysis. It is the objective of this study to understand how this accelerometer-like system is capable of complex signal analysis and processing. More specifically, we intend to determine auditory capabilities and mechanisms of fishes, the contributions of various parts of the auditory system (ear and peripheral structures) to signal analysis, and the neuroanatomy ('wiring') of the auditory portion of the CNS as it might contribute to signal analysis.

OBJECTIVES

The P.I.'s, in collaboration with Dr. Joelle Presson and Dr. Hong Y. Yan, have been attempting to understand the structure and function of the inner ear of fishes by performing a series of studies to analyze the structure and neural connectivity in the ear. We investigated the sites at which spikes are initiated in the ear epithelia and the regionalization of neuronal innervation patterns. We are also considered questions about the kinds of sounds fishes can detect and, ultimately, the processing of these sounds at different levels of the auditory system.

SUMMARY OF MAJOR RESULTS

A. Structure and Innervation of the Ear

- investigated the structure of the ear in a primitive fish, the gar (*Mathiesen & Popper, 1987*)
- analyzed the afferent and efferent innervation of the saccule (*Popper & Saidel, 1990*)
- analyzed innervation patterns of eighth nerve afferent neurons to the saccule and lagena in

several species (*Presson et al., 1992*)

- ♦ determined sites of spike initiation for different epithelial regions in three otic endorgans (*Saidel, 1988; Saidel et al., 1990a*)
- ♦ demonstrated that there are physiological differences in sensory cells in different regions of the ear (*Saidel et al, 1990b; Yan et al., 1991*)
- ♦ discovered that the fish hear has multiple types of sensory hair cells (*Chang et al, 1991; in preparation; Popper et al, in preparation*)
- ♦ Demonstrated that sensory hair cells of the ear can be regenerated after trauma (*Yan et al., 1992; Lombarte et al., in preparation*)
- ♦ developed a model for the fish auditory system that continues to serve as an excellent guide for understanding how fish might process sounds (*Popper et al., 1988; Rogers et al., 1988; Schellart & Popper, 1992*)

B. Fish Hearing Capabilities

- ♦ determined hearing capabilities in fishes that have specializations as compared to fishes that do not have hearing specializations (*Saidel & Popper, 1987*)
- ♦ developed a new method for determining hearing capabilities of fishes (*Yan & Popper, 1991*).
- ♦ determined hearing capabilities of a fish that is not considered a hearing specialist (*Yan & Popper, in preparation*)

OVERVIEW OF MAJOR RESULTS

Our investigations over the past several years have involved two major thrusts. One has been to understand the structure and function of the ear and the second to provide insight into hearing capabilities of fish.

Model of Fish Hearing

In our investigations of the structure and function of the ear, several major results have occurred that provide the potential for significant changes in how we view the fish, and vertebrate, auditory system. In a close collaboration with Dr. Peter Rogers (Georgia Tech) and Dr. Mardi Hastings (now at Ohio State), we developed a model for the fish auditory system that has not only served to help us understand the ways that fish might process sound, but has also served as a predictor of results. Specifically, the model successfully predicted that fish must add sensory hair cells throughout their lives, which is precisely what has been found in other, unrelated, work in our laboratory (supported by NIH). The model indicates that fishes require information from both a pressure receptor (the swimbladder) and acceleration detector (directly via the inner ear) to determine sound source direction, distance, and frequency. Current studies are designed to test at least part of this hypothesis.

Structure of the Fish Ear

A number of studies in our laboratory have been directed at understanding the structure of the ear. We have used a variety of different techniques, including immunocytochemistry, neuroanatomy and treatment with ototoxic drugs to analyze sensory hair cells of the ear. Each of these studies (Saidel 1988; Saidel et al 1990a, b; Yan et al, 1991) suggested that there are different 'classes' of receptors in the fish ear, and particularly in the saccule.

Immunoreactivity to S-100 and Calmodulin: We tested the immunoreactivity of hair cells to a variety of antibodies and have focused on two antibodies to antigens involved in calcium physiology. (Calcium is intimately involved in membrane processes that affect the biophysical signal, from the transduction step to the synaptic step.) S-100 and calmodulin are two proteins that sequester calcium. A commercial antibody to S-100 and an anti-calmodulin were tested for reactivity to hair cells in the fish ear. The antibody to S-100 selectively stains the cytoplasm of a population of hair cells in both the utricle and the saccule. The stained hair cells are in the striola of the utricle and in the central region of the saçcule. The two regions have in common the hair cells with short ciliary bundles.

Likewise, the anti-calmodulin stained the same cell types in similar locations. However, the antibody stained a different cellular compartment, the nucleus. Thus, with two different measures, we have produced evidence that hair cells are biochemically different in a manner that might relate to signally properties.

Response to an ototoxic drug: We have recently discovered that a drug, gentamicin, selectively damages sensory hair cells in some parts of the ear and not in others. This drug is known to be selectively ototoxic in mammals, and it apparently has the same affect in fish. Cells damaged by gentamicin are the same cells that are stained by S-100 (see above) and also the same cells that have neurons that react to a ferric-ferrocynaide stain (progress report last year). The combination of these three results strongly suggest differentiation in hair cell function in the ear. We have also found that the hair cells that are destroyed by gentamicin are regenerated within several days, demonstrating for the first time that fish are capable of regenerating sensory hair cells as well as proliferating hair cells.

Ultrastructure of sensory hair cells: Most recently, ultrastructural studies (Chang & Popper, 1991; Chang et al, in preparation) have made the major discovery that the hair cells that respond to S-100 and gentamicin, and that have the very large neurons, are also ultrastructurally very distinct from hair cells that do not respond to these various treatments. *Most importantly*, these hair cells, which are located in the striolar region of the utricle, closely resemble type I cells of amniotes, while the other utriclar cells resemble amniote type II cells. This discovery is important for two reasons. First, we have demonstrated, for the first time, that fish ears have two distinct classes of receptor cells, indicating that the ear processes information in two different ways. Secondly, all previous studies have categorically stated that fishes do not have type I hair cells, and that such hair cells are newly evolved with amniotes. While we cannot say that our striolar hair cells are precisely the same as amniote type I cells, they are sufficiently similar that we can suggest that the type I cells of amniotes are *not unique*, and that they may have evolved over 200 million years earlier than previously thought. We have named these new cells type I-like cells.

We have also gone back to examine tissue from earlier studies and have now found that type I-like and type II cells also occur in the saccule of the oscar. This reinforces our suggestion that there are hair cells with different functions in the fish ear, and that each of the otolith organs performs multiple roles in hearing and in dealing with gravistatic information.

Innervation of the ear

Eighth nerve neurons: In the oscar, there are clear regional differences in the innervation of the saccular sensory epithelium, the primary inner ear end organ for audition. Small diameter axons with small terminal arbors innervate all parts of the epithelium including the periphery. Very large diameter axons, with small diameter terminals, are confined to the rostral end of the saccule. Finally, medium sized axons with larger terminal arbors are confined to the center of the saccule, all along its rostro-caudal extent.

The eighth nerve axons innervating the saccule in the goldfish are not different from those in the oscar with respect to axon diameter. However, our sample of axons from the goldfish does **not** include any with very large arbor diameters, while in the oscar these large axons comprise over 15% of the sample. Furthermore, the regional differences encountered in the oscar saccule are not apparent in the goldfish saccule. These findings suggest that there may be inherent differences in the information processing strategies in the saccules of the two species, although more data are needed before we can accept this hypothesis.

One of the most significant aspects of the fish auditory system is that the end organs of the ear continue to grow, and add sensory hair cells, throughout the life of the fish (Popper & Hoxter, 1984). Thus, we have been asking how addition of hair cells affects the 'wiring' of the ear and, ultimately, the way in which the fish deals with auditory and vestibular stimuli. We are currently studying the innervation of the saccule in small and large oscars. At this point in the analysis we have found that large oscars have larger eighth nerve arbors than do small animals. A more detailed of arbor morphologies will be completed in the near future.

Innervation of sensory hair cells of the ear: We reconstructed, using serial section electron microscopic analysis, sensory hair cells at different epithelial regions in order to determine synaptic structure and synaptic positions in different sensory hair cells. Each of the hair cells analyzed (N=67) has from 1 to 14 afferent synapses and 0 to 14 efferent synapses. A single hair cell may make several synapses with one or more afferent nerve fibers, while each fiber must also innervate multiple hair cells. The number of each synapse type, and the ratio of afferent to efferent synapses, varies substantially among hair cells. The ratio of afferent to efferent synapses ranges from 0.25:1 to 7.0:1. Interestingly on nearly every hair cell examined, efferent synapses are located along the length of each cell in a band extending from the top of the nucleus to the base of the cell, while afferent synapses are restricted to a narrower band within the efferent band.

The results from our serial analysis of hair cells demonstrate differences in synaptic patterns of hair cells in different epithelial regions and even between neighboring hair cells. The profile variations in synaptic descriptions of hair cells, even within restricted macular regions, will, we suspect, result in response differences among eighth nerve axons. The broad spectrum of innervation patterns encountered with different hair cells suggests variation of peripheral computation of stimulus information. These data are useful for refining the model in that the data provide specific information on the 'wiring' of the sensory hair cells.

Hearing by Fish

Behavioral Analysis of hearing: In addition to asking questions about the structure and innervation of the year, it is important to determine hearing capabilities of fishes, with the ultimate goal of testing hypotheses regarding inner ear function. Previous work in our lab and others has used various procedures that involved aversive stimuli (usually shock) to train fish. Our feeling has been that such procedures are potentially harmful to the animals and may do other damage. Consequently, we have developed the first method for measuring hearing capabilities of fishes using a reward procedure. This procedure involves giving fish a food reward for a correct response when a sound is present. In order to insure that the technique is valid, we have successfully compared data on hearing capabilities determined using this technique to results from earlier techniques and our data are comparable. This procedure will be used for determination of other aspects of fish hearing.

Most recently, we have completed an audiogram for the oscar, our major experimental species. This species does not have specializations for hearing (as does the goldfish, the species with which we developed this technique) and we found that it can only hear from 200-600 Hz, with relatively poor sensitivity.

Comparison of hearing in hearing a hearing specialist vs a non-specialist: Using microphonic potentials to record from the ears of two species of anabantid fish, we determined that fishes that have a bubble of air in the pharyngeal cavity (close to the ear) hear substantially better than fishes without such an adaptation. The hearing specialist had a wider bandwidth and substantially better sensitivity than the non-specialist.

SIGNIFICANCE OF ONR INVESTIGATIONS

Our investigations supported by the ONR contract have allowed us to make significant contributions to our understanding of fish hearing in general, and to the structure and function of the fish ear. This is particularly significant since the fish ear is, in effect, a small accelerometer-like system that is capable of detection and analysis of highly complex signals.

Our model of the fish auditory system has served as a general guide to studies of hearing. While we are confident that the model will have to be refined in the future, it does provide the only available model in the literature that is capable of accounting for how fishes detect sounds, determine sound source direction, and shows how fishes can detect and analyze sound frequency and detect signals in the presence of noise. Most importantly, our model can account for detection and processing of all types of sounds, from pure tones to more biologically significant noises and complex signals. In contrast, all other models can only deal with pure tones, a type of signal that is rarely encountered by fish.

Our experimental studies have involved a series of investigations using a wide range of techniques to understand the structure of the ear, and particularly of the sensory hair cells. These studies have lead to our most recent, and important, finding that there are two very distinct classes of receptor cells in the fish ear. While the function of the two types of cells are not yet understood, functional differences in analogous amniote cells suggest to us that there may be fundamental differences in the ways the two cells in the fish ear function. We can only speculate at this point, but one possible explanation for the two types of receptors is that each serves as a filter for different aspects of a signal. For example, the type II cells, by the nature of their locations in the various endorgans, may be responsive primarily to low frequency signals while type I-like cells may be for the detection of higher frequencies.

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